A newly developed hydrophilic polymer-based ion exchange chromatography media and purification of Immunoglobulin Y from egg yolk

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Introduction

The recent development of bio-pharmaceutical industry has been remarkable, and shortening the development time and reducing the cost become increasingly important. The development of efficient, economical and selective separation method is required for successful commercialization of bio-pharmaceutical products. To meet these demands, we have developed new polymeric packing materials named YMC-BioPro series, which are specially designed for ion exchange (IEX) separation and purification of proteins, peptides and nucleic acids. YMC-BioPro series includes the packed columns with 5 micron porous/non-porous polymer for analysis and laboratory scale purification, and the bulk materials of 30, 75 micron porous polymer for capture and intermediate purification. The all materials are based on the same hydrophilic polymer beads with low nonspecific adsorption.

In this poster, we show the properties and benefits of these new IEX chromatography media, including separation selectivity of some important biomolecules such as monoclonal antibody and DNA, binding capacity and recovery at various linear velocity, and chemical stability under cleaning-in-place condition with 1.0 M NaOH. We also present the efficient purification protocol of Immunoglobulin Y (IgY) from egg yolk.

Features of new IEX chromatography media

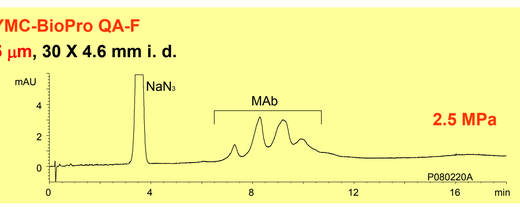
- Newly developed hydrophilic polymer with low nonspecific adsorption
- Porous polymer beads with high binding capacity and high recovery of biomolecules
- Non-porous polymer beads with high chemical and mechanical stabilities
- 5 μm packed column for high-resolution and high-throughput analysis, or laboratory-scale purification
- 30, 75 μm porous bulk materials for capture and intermediate purification

	Pre-Packed Columns		Bulk materials	
	YMC-BioPro QA-F/SP-F	YMC-BioPro QA/SP	YMC-BioPro Q30/S30	YMC-BioPro Q75/S75
Particle size (μm)	5		30	75
Matrix	non-porous polymer beads	porous polymer beads	porous polymer beads	
Charged group	QA-F/QA : $-CH_2N^+(CH_3)_3$ SP-F/SP : $-(CH_2)_3SO_3^-$		Q30/Q75 : $-CH_2N^+(CH_3)_3$ S30/S75 : $-(CH_2)_4SO_3^-$	
Dynamic binding capacity (mg/ml-resin)	QA-F : >12 (BSA) SP-F : >10 (IgG)	QA : >110 (BSA) SP > 70 (IgG)	Q30/Q75 : >160 (BSA) S30/S75 : > 160 (Lysozyme)	
Available pH range	2～12		2～12	

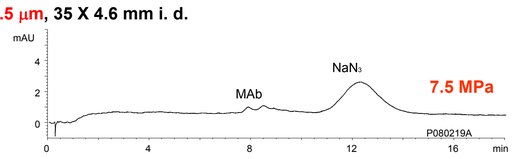
Comparison of selectivity and resolution on non-porous/porous IEX columns

Analysis of IgG1 monoclonal antibody (MAb) on non-porous type anion-exchange columns

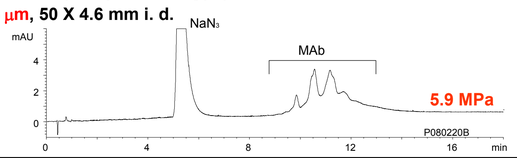
YMC-BioPro QA-F
5 μm, 30 X 4.6 mm i. d.



Brand T (non-porous DEAE type)
2.5 μm, 35 X 4.6 mm i. d.



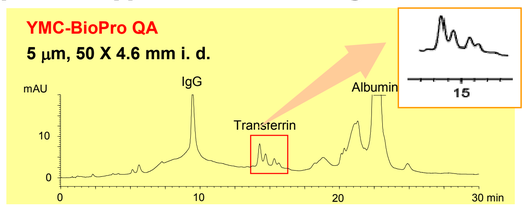
Brand G (non-porous Q type)
3 μm, 50 X 4.6 mm i. d.



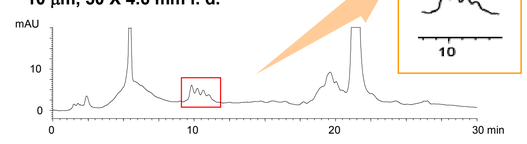
Eluent	: A) 20 mM Tris-HCl (pH 8.1) B) 20 mM Tris-HCl (pH 8.1) containing 0.5 M NaCl 10-25%B (0-18 min)
Flow rate	: 1.0 ml/min (180 cm/hr)
Temperature	: 25℃
Detection	: UV at 220 nm
Injection	: 10 μl (0.1 mg/ml)
Sample	: Monoclonal mouse IgG1 against human IgG4 (Purified by DEAE chromatography, containing NaN_3)

Analysis of proteins in human serum on porous type anion-exchange columns

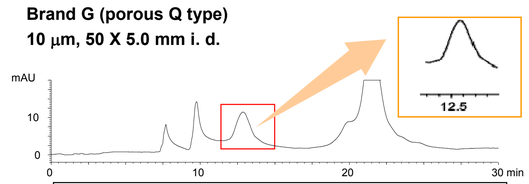
YMC-BioPro QA
5 μm, 50 X 4.6 mm i. d.



Brand T (porous Q type)
10 μm, 50 X 4.6 mm i. d.



Brand G (porous Q type)
10 μm, 50 X 5.0 mm i. d.



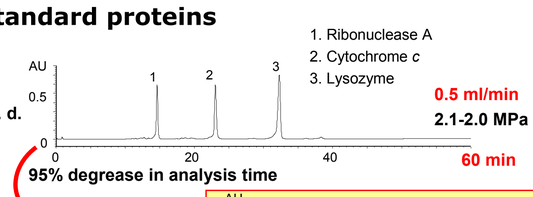
Eluent	: A) 20 mM Tris-HCl (pH 8.6) B) 20 mM Tris-HCl (pH 8.6 containing 0.5 M NaCl 0-30%B (0-15 min), 30-100%B (15-30 min)
Flow rate	: 0.5 ml/min
Temperature	: 25℃
Detection	: UV at 280 nm
Injection	: 20 μl
Sample	: Human serum (100 μl/ml)

The separation of biological samples is compared on various commercially available IEX columns in analytical scale. As shown in the left figures, non-porous type YMC-BioPro QA-F column can achieve superior resolution of MAb in less analysis time at lower back pressure than two other columns which are packed with smaller particles. The right figures show that porous type YMC-BioPro QA is suitable for high-resolution analysis of protein samples containing a large amount of impurities. The optimal packed 5 μm spherical monodispersed beads with proprietary surface modification provides high efficiency and high selectivity to YMC-BioPro columns.

High-throughput analysis on non-porous short column with higher flow rate

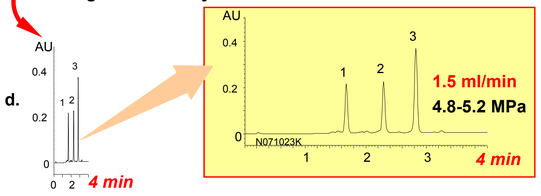
Analysis of standard proteins

Porous type
YMC-BioPro SP
5 μm, 50 X 4.6 mm i. d.



95% degrease in analysis time

Non-porous type
YMC-BioPro SP-F
5 μm, 30 X 4.6 mm i. d.



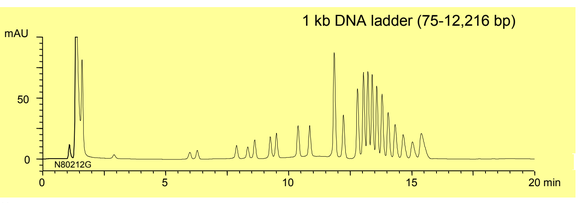
Eluent	: A) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) B) 20 mM KH_2PO_4-K_2HPO_4 (pH 6.8) containing 0.5 M NaCl 0-100%B (0-4 min) for YMC-BioPro SP-F 0-100%B (0-60 min) for YMC-BioPro SP
Temperature	: 25℃
Detection	: UV at 220 nm
Injection	: 20 μl

The high mechanical stability of non-porous polymer beads enables faster elution of proteins with higher flow rate. The 30 mm and 50 mm-length column of YMC-BioPro QA/F/SP-F is designed for high-throughput analysis with low operating pressure.

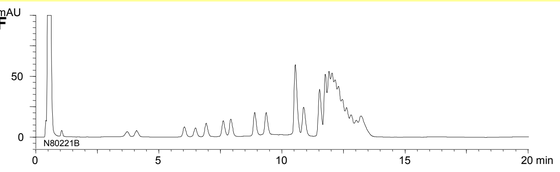
High-resolution analysis on non-porous 100 mm-length column

Analysis of DNA fragment

YMC-BioPro QA-F
100 X 4.6 mm i. d.



YMC-BioPro QA-F
30 X 4.6 mm i. d.

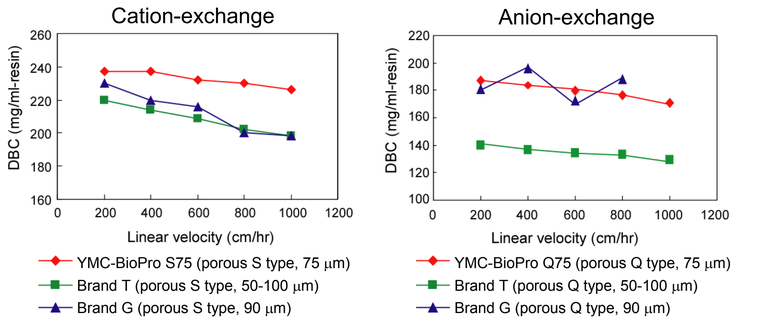


Eluent	: A) 20 mM Tris-HCl (pH 8.1) containing 0.7 M NaCl B) 20 mM Tris-HCl (pH 8.1) containing 1.0 M NaCl 0-100%B (0-30 min)
Flow rate	: 0.5 ml/min (180 cm/hr)
Temperature	: 25℃
Detection	: UV at 260 nm
Injection	: 20 μl (0.25 mg/ml)

The separation of DNA fragments is compared between 100 mm-length and 30 mm-length of YMC-BioPro QA-F columns. The resolution of DNA fragments is dramatically improved by 100 mm column.

The combination of non-porous polymer beads and long column length provides extremely high column efficiency.

Comparison of dynamic binding capacity (DBC) at different flow rate on IEX resins for capture and intermediate purification



Column size	: 50 X 5.0 mm i. d.
Sample	: 1.0 mg/ml Lysozyme in equilibration buffer
Equilibration buffer	: 20mM Glycine-NaOH (pH 9.0)
Elution buffer	: 20mM Glycine-NaOH (pH 9.0) containing 0.5 M NaCl
Detection	: UV at 300 nm

Column size	: 55 X 5.0 mm i. d.
Sample	: 1.5 mg/ml BSA in equilibration buffer
Equilibration buffer	: 20mM Tris-HCl (pH 8.6)
Elution buffer	: 20mM Tris-HCl (pH 8.6) containing 0.5 M NaCl
Detection	: UV at 280 nm

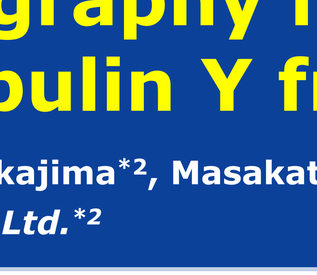
The dependency of DBC to linear velocity is compared on various commercially available IEX resins for capture and intermediate purification. YMC-BioPro S75 and Q75 shows the high DBC over a wide range of linear velocity, and the difference of DBC is only less than 5% between 200 cm/hr and 1000 cm/hr.

Use of IEX resins at flow rates of up to 1000 cm/hr at high DBC enables rapid large scale purification. This shows 75 μm BioPro resins would give increased productivity and reduced cost in biopharmaceutical production.

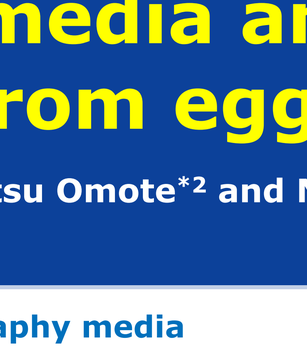
Cleaning-in-place (CIP) study of BioPro S75 resin

Test protocols

DBC and recovery



Separation of standard proteins



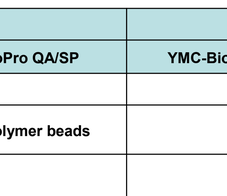
Column	: YMC-BioPro S75, 50X5.0 mm i. d.
Conditions of DBC determination	
Equilibration buffer	: 20 mM Glycine-NaOH (pH 9.0)
Elution buffer	: 0.5 M NaCl in equilibration buffer
Flow rate	: 2.62 ml/min (800 cm/hr)
Sample	: 1.0 mg/ml Lysozyme in equilibration buffer
Temperature	: ambient (25℃)
Detection	: UV at 300 nm
※DBC is determined at 10% breakthrough.	
Conditions of protein separation	
Eluent	: A) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) B) 20 mM NaH_2PO_4-Na_2HPO_4 (pH 6.8) containing 0.5 M NaCl
Gradient	: 0-100%B (0-60 min, Linear)
Flow rate	: 0.59 ml/min (180 cm/hr)
Temperature	: 25 ℃
Detection	: UV at 220 nm
Injection	: 24 ul

The DBC and the selectivity of protein separation are unaffected following 20 cycles of CIP with 1.0 M NaOH. The high chemical stability of BioPro resins allow effective cleaning with alkaline solution. The recovery is maintained at a constant value around 100%. These shows the hydrophilic properties of the matrix polymer remarkably reduce nonspecific adsorption of proteins.

Two step purification of IgY from egg yolk extract

***Crude extract from egg yolk containing IgY**

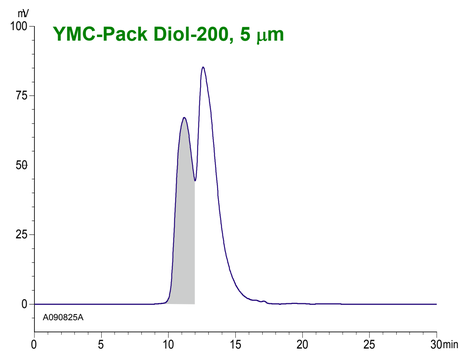
*Crude extract is the supernatant after precipitation of the lipids and lipoproteins from egg yolk.



***Step 1*:**
Capture purification by ion-exchange chromatography (IEC)

Sample	: Crude extract from egg yolk
Column	: YMC-BioPro Q75, 75 μm, 50 X 4.6 mm i. d.
Eluent	: A) 20mM Tris-HCl (pH 8.1) B) 20mM Tris-HCl (pH 8.1) containing 0.5M NaCl 10%B (0-15 min), 30%B (15-30 min), 90%B (30-40 min)
Flow rate	: 0.5 ml/min (180 cm/hr)
Temperature	: ambient
Detection	: UV at 280 nm
Injection	: 1 ml (approx. 20 mg protein)

***Step 2*:**
Final purification by size-exclusion chromatography (SEC)



Sample	: Fraction from capture purification by IEC
Column	: YMC-Pack Diol-200, 5 μm, 300 X 8.0 mm i. d.
Eluent	: 0.1M KH_2PO_4-K_2HPO_4 (pH 6.9) containing 0.2 M NaCl
Flow rate	: 0.7 ml/min (180 cm/hr)
Temperature	: ambient
Detection	: UV at 280 nm
Injection	: 1 ml (approx. 0.45 mg IgY)

Analysis of crude and purified fraction

Column	: YMC-Pack Diol-200 5 μm, 300 X 4.6 mm i. d.
Eluent	: 0.1M KH_2PO_4-K_2HPO_4 (pH 6.9) containing 0.2 M NaCl
Flow rate	: 0.23 ml/min
Temperature	: ambient
Detection	: UV at 280 nm

Egg yolk antibody, IgY, is purified by two chromatographic steps using IEC as a capture purification and SEC as a final purification. Purity of the IgY is checked with SDS-PAGE and SEC analysis shown below.

This purification protocol can isolate IgY with high purity more than 99%.

Conclusions

- Using the optimal IEX material for a specific application can result in a significant decrease in the costs of the biopharmaceutical production. YMC-BioPro series, which based on the same hydrophilic polymer with low non-specific adsorption, is scalable from analytical to large-scale preparative separation.
- 5 μm pre-packed columns with optimal packing technology provide superior resolution. Non-porous type BioPro QA-F/SP-F columns are effective for high resolution analysis or QC assessment of complex mixtures, such as MAbs. Porous type BioPro QA/SP columns are useful for analysis and laboratory-scale purification of biological samples.
- 30 μm and 75 μm bulk materials of porous polymer are useful for high capacity capture and high efficiency intermediate purification steps.